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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/697,991	10/30/2003	M. Benjamin Perryman	UTEC:007US	7402

7590 10/19/2004

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EXAMINER	
GAKH, YELENA G	
ART UNIT	**PAPER NUMBER**
1743	

DATE MAILED: 10/19/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/697,991	PERRYMAN ET AL.	
	Examiner	Art Unit	
	Yelena G. Gakh, Ph.D.	1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 October 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-52* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-52* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *03/22/04*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claim Rejections - 35 USC § 112

1. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 13 and 25 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 13 and 25 recite the limitation "said source". There is insufficient antecedent basis for this limitation in the claim, as no source is recited in the parent claims.

Claim Rejections - 35 USC § 102

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

4. **Claims 1-2, 4, 6, 8, 11, 12, 13, 18-28, 30, 32, 34, 37-39, 44-52** are rejected under 35 U.S.C. 102(a) or 102(e) as being anticipated by Tubbs et al. (US 2002/0094566 A1).

Tubbs teaches MALDI-TOF quantitative analysis of complex protein mixtures in biological samples using internal standards (IS), with the following examples of the IS indicated: "internal reference standards that behave similarly to the analyte during laser desorption/

ionization are generally preferred. This prerequisite is met during MSIA by choosing internal references that share sequence homology with the target protein: enzymatic/chemically-modified versions of the targeted protein, truncated/extended recombinant forms of the target proteins, the (same) target protein recombinantly expressed in isotopically-enriched media (e.g., ^{15}N or ^{18}O), or the same protein from a different biological species" (page 16, [0157]). "As used herein, "biological media" or "biological sample" refers to a fluid or extract having a biological origin. Biological media may be, but are not limited to, cell extracts, nuclear extracts, cell lysates and excretions, blood, sera, plasma, urine, sputum, sinovial fluid, cerebral-spinal fluid, tears, feces, saliva, membrane extracts, and the like" (page 22, [0202]). "FIG. 17b Spectrum in (2) shows EDTA/Ca.sup.2+ affinity pipette capture of two phosphate rich proteins, PRP-1 and PRP 3. Mass signature of dephosphorylation is evident in spectral trace (3) and complete in (4). Illustrating multi-analyte detection accompanied by partial and complete dephosphorylation of phospho-proteins captured/digested out of biological fluid for post-translational analysis (i.e., phosphorylation events" (isoforms in form of phosphoisomers) (page 4, [0038]).

5. **Claims 1-2, 6, 8, 24-25, 30-32, 51 and 52** are rejected under 35 U.S.C. 102(b) as being anticipated by Mirgorodskaya et al. (Rapid Comm. Mass. Spectrom., 2000).

Mirgorodskaya teaches "quantitation of peptides and proteins by matrix-assisted laser desorption/ionization [MALDI] mass spectrometry using ^{18}O-labeled internal standards" (Title). "The biopolymer cleavage reaction is performed under the same conditions as the reaction of the standard, with the only difference that the product is prepared in $H_2{}^{16}O$, while the internal standard is prepared in $H_2{}^{18}O$. ... The initial biopolymer concentration can be calculated by the analysis of the isotopic pattern resulting from ^{18}O-labeled internal standard which overlaps with the natural isotopic distribution of the peptide selected for quantitation" (page 1226, right column)". Quantitation of proteins by MALDI using ^{18}O-modified proteins as internal standards are described on pages 1229-1231.

6. **Claims 1-6, 8, 11-13, 18-25, 28-32, 34, 37-39, 44-52** are rejected under 35 U.S.C. 102(b) as being anticipated by Lahm et al. (Electrophoresis, 2000).

Lahm reviews "mass spectrometry: a tool for the identification of proteins separated by gels". He specifically teaches "quantification of proteins": "bacterial or eukariotic cells are grown on a media containing ^{15}N as sole nitrogen source, and then the cells grown under these

conditions are mixes with cells grown under normal conditions. The same amount of labeled cells is added to the control (no treatment) as to the cells, which were treated or are in a different physiological state (shown in Fig. 1). This mixture is applied to 2-D gels and the spots are excised and cleaved with trypsin. The ratio of the areas under the peaks derived from the ^{14}N-peptides and from the ^{15}N-peptids is calculated (Fig. 2) for several peptides from each protein spot. The same procedure is followed for both the untreated and the treated. When the ratio has changed from the treated to the treated cells one gets an accurate value of the change factor" (page 2110). On page 2106 Lahm indicates, "MALDI-TOF-MS is one of the most widely used types of instrument for protein identification from 2-D gels". Various digestion techniques are disclosed on page 2108.

7. **Claims 1-2, 4, 6, 8, 13, 18-25, 28, 30, 32, 34, 39, 44-52** are rejected under 35 U.S.C. 102(b) as being anticipated by Goborn et al. (Anal. Chem., 2000).

Goborn teaches "detection and quantification of neurotensin [NT] in human brain tissue by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry" using stable-isotope-labeled NT (Abstract). "Quantificaiton of the known biologically active form of the peptide NT 1-13 was achieved by spiing the crude tissue extracts with synthetic stable-isotope-labeled NT in which six carbon atoms in Leu were replaced by ^{13}C [$^{13}C_6$]NT), giving a molecular mass increment of 6 Da. The use of a standard that is chemically equivalent to the target molecule eliminates errors due to different affinities of the antigen and standard for the antibody and different ionization efficiencies in the MALDI process" (page 3321, right column).

8. **Claims 1-2, 4, 6, 8, 13, 18-25, 28, 30, 32, 34, 39, 44-52** are rejected under 35 U.S.C. 102(a) as being anticipated by Kachman et al. (Anal. Chem., April 15, 2002).

Kachman discloses "a 2-D liquid separations/mass mapping method for interlysate comparison of ovarian cancers" (Title), with mapping over 50 proteins; the mapping allows differentiation between posttranslational protein modifications, such as phosphorylation and acetylation (page 1779, right column). "The critical consequence of gene mutations is altered protein expression within cancer cells, which may be reflected in expression of new proteins, differences in the amount of expressed proteins, and differences in posttranslational protein modifications, such as phosphorilation and acetylation" (page 1779, right column). For

quantitative MALDI-MS analysis angiotensin I, adrenocorticotropin (ACTH, amino acids 1-17), ACTH (18-39), and ACTH (7-38) were added (page 1783, right column).

9. **Claims 1-2, 4-6, 8, 13, 18-25, 28, 30-32, 34, 39, 44-52**are rejected under 35 U.S.C. 102(a) as being anticipated by Bucknall et al. (J. Am. Soc. Mass Spectrom., September 2002, IDS).

Bucknal teaches "the practical utility of automated MALDI-TOFMS as a tool for quantifying a diverse array of biomolecules covering an extensive molecular weight range, and present in biological extracts and fluids. Growth hormone was measured in rat pituitary tissue; insulin in human pancreatic tissue; homovanillic acid in human urine; and LVV-hemorphin-7, epinephrine and norepinephrine in human adrenal and pheochromocytoma tissues. Internal standards including compounds of similar molecular weight, structural analogs or isotopomers were incorporated into each analysis" (Abstract).

Claim Rejections - 35 USC § 103

10. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

12. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out

the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

13. **Claims 7, 9-10, 14-15, 18-23, 29-36, 40-41 and 44-49** are rejected under 35 U.S.C. 103(a) as being unpatentable over any of the references cited above in view of Forssmann et al. (US 6,326,163 B1).

The references cited above do not specifically teach quantification of α- or β-myosin heavy chain by MALDI-TOF.

Forssmann teaches "a quick method for the qualitative and quantitative medical-diagnostic analysis on the protein level of the substitution of single amino acids with pathogenic and non-pathogenic effects on the organism. The medical-diagnostic analysis is performed by a combination of enzymatic or chemical cleavage of the isolated peptide, chromatographical separation of the fragments and analysis by mass spectrometry, both direct LC/MS and indirect MALDI-MS, and analysis by capillary electrophoresis. By comparing protein samples from healthy humans with those of ill humans, the method described is suitable for establishing new, as yet unknown mutations and quantifying the expression and incorporation of wild type to mutant" (Abstract). The invention is "illustrated by using the heavy chain of ß-isoform of myosin as an example. Point mutations in the heavy chain of ß-isoform of myosin, e.g., substitution of the amino acid methionine for the amino acid valine in position 606, may result in hypertrophic cardiomyopathy, a genetically caused thickening of certain heart walls, which may lead to sudden death. According to the invention, the detection of the mutation is possible by a combination of enzymatic cleavage and LC/MS. FIGS. 1a and 1b show the analysis of the peptide fragments of human cardiac ß -myosin heavy chain (ß -MHC) by means of a coupling of high performance liquid chromatography (HPLC) with mass spectrometry (MS). In this example, the presence of the heterozygotic mutant Val606Met, i.e., substitution of valin in position 606 of ß-MHC, was looked for. FIG. 1a shows two marked ranges in which the fragment with a substituted amino acid and a molecular weight of 1507.5 could be detected in addition to the original fragment, the wild type fragment having a molecular weight of 1475.5, in a person for whom the substitution had been proven on the gene level. For comparison, FIG. 1b shows the analysis of a ß -MHC sample of a person with no point mutation in this gene. In this

case, only the wild type fragment can be detected; in the range in which the mutated fragment was eluted in FIG. 1a, this peptide is completely lacking. Thus, it becomes possible to detect mutated ß-MHC in a person's muscle fibers" (col. 3, lines 1-30).

It would have been obvious for anyone of ordinary skill in the art to apply the method of quantitative measurement of proteins, including their mixtures, by MALDI-TOF analysis using internal standards, as disclosed in the references cited above, to α- or β-myosin isoforms heavy chains, because Forssmann teaches the importance of quantitative measurements of these proteins for monitoring cardiovascular diseases, and the method disclosed by the references cited above allows to do this in a most efficient way.

14. **Claims 16-17 and 42-43** are rejected under 35 U.S.C. 103(a) as being unpatentable over any of the references cited above in view of England et al. (Cellular Signalling, 2002).

The references cited above do not specifically teach quantification of cardiac or skeletal actin by MALDI-TOF.

England teaches, "proteins coimmunoprecipitating with protein kinase C (PKC) epsilon in fibroblasts were identified through matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI TOF m/s). This method identified myosin IIA in PKC epsilon immunoprecipitates, as well as known PKC epsilon binding proteins, actin, beta'Cop and cytokeratin" (Abstract).

It would have been obvious for anyone of ordinary skill in the art to apply the method of quantitative measurement of proteins, including their mixtures, by MALDI-TOF analysis using internal standards, as disclosed in the references cited above, to actin, because England indicates the importance of actin as a modulator of PKC activity, which is essential for cellular growth.

Conclusion

15. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. *Tang et al. (Anal. Chem., 1996)* teach "detection and quantitation of ß-2-microglobulin glycosylated end products in human serum by matrix-assisted laser desorption/ionization [MALDI] mass spectrometry" (Title): "In an attempt to study the possibility of quantitation by MALDI the peak areas of $ß_2$M-AGEs were correlated with the

concentrations of these species in human serum standards. Pulse-to-pulse laser energy variations and sample inhomogeneity necessitated the use of internal standards for quantitative studies. Throughout the study $ß_2M$ was used as an internal standard to normalize the $ß_2M$-AGE signals" (page 3742, left column). *Sarto et al. (Electrophoresis, 1999)* teach "modified expression of plasma glutathione peroxide and manganese superoxide dismutase in human renal cell carcinoma" (Title) with identifying two isoforms of the two first peptides by MALDI-TOF-MS and internal sequence analysis; *Hernandez et al. (Glycoconjugate J., 2001)* disclose "chemical characterization of the lectin from *Amaranthus leucocarpus syn.* hypocondriacus by 2-D proteome analysis" with determining three isoforms by MALDI-TOF of trypsin digests.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yelena G. Gakh, Ph.D. whose telephone number is (571) 272-1257. The examiner can normally be reached on 9:30 am - 6:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill A. Warden can be reached on (571) 272-1267. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Yelena G. Gakh
10/8/04